

What is claimed is:

1. A method for improving a genetic stability of a foreign insert nucleotide sequence in a recombinant single-stranded RNA virus vector, which comprises performing a mutagenesis of the foreign insert nucleotide sequence to provide even distribution of G/C content throughout the overall foreign DNA sequence.
2. The method according to claim 1, wherein the recombinant single-stranded RNA virus vector is derived from one selected from the group consisting of a poliovirus recombinant vector, a yellow fever virus vector, a Venezuelan equine encephalitis virus vector, a rubella virus vector and a Cocksackie virus vector.
3. A method for improving a genetic stability of a foreign insert nucleotide sequence in a recombinant poliovirus vector, which comprises performing a mutagenesis of the foreign insert nucleotide sequence to provide even distribution of G/C content throughout the overall foreign DNA sequence.
4. The method according to claim 1 or 3, wherein the mutagenesis to provide even distribution of G/C content throughout the overall foreign DNA sequence is performed by increasing G/C content of the foreign DNA sequence.

5. The method according to claim 4, wherein the mutagenesis renders the foreign DNA sequence to have the G/C content of more than 40%.

5 6. The method according to claim 1 or 3, wherein the mutagenesis of the foreign DNA sequence to provide even distribution of G/C content is performed by increasing G/C content of local A/T-rich region in the foreign DNA sequence.

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7. The method according to claim 1 or 3, wherein the mutagenesis is performed by silent mutations.

8. The method according to claim 1 or 3, wherein the
15 foreign DNA sequence is smaller than 450 bp in size.

9. The method according to claim 2 or 3, wherein the recombinant poliovirus vector is one selected from the group consisting of poliovirus type 1, poliovirus type 2
20 and poliovirus type 3.

10. The method according to claim 2 or 3, wherein the poliovirus is one selected from the group consisting of Sabin type 1, Sabin type 2 and Sabin type 3.

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11. The method according to claim 1 or 3, wherein the foreign DNA sequence encodes an antigenic determinant site

of an infectious virus selected from HIV, HCV, HBV, HPV, HSV, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

5 12. The method according to claim 3, wherein the foreign DNA sequence is inserted into the 5'-end of the recombinant poliovirus vector.

13. The method according to claim 12, wherein the foreign
10 DNA sequence is inserted into a cloning site containing at least one restriction cleavage site artificially formed at the 5'-end of the recombinant poliovirus vector.

14. The method according to claim 13, wherein the cloning
15 site further comprises at its 3'-direction a protease cleavage site recognized by 3C-protease encoded by a poliovirus genome.

15. A method for improving a genetic stability of a
20 foreign insert nucleotide sequence in a recombinant Sabin type 1 poliovirus vector comprising (a) a genomic nucleotide sequence of poliovirus; (b) a cloning site containing restriction cleavage sites artificially formed at the 5'-end of the genomic nucleotide sequence; and (c)
25 a 3C-protease cleavage site formed between the genomic nucleotide sequence and the cloning site, which comprises performing a mutagenesis of the foreign DNA sequence

inserted into the cloning site in order to provide even distribution of G/C content throughout the overall foreign DNA sequence and to have the G/C content of more than 40%.

5 16. A method for improving a genetic stability of a foreign insert nucleotide sequence in a recombinant Sabin type 1 poliovirus vector comprising (a) a genomic nucleotide sequence of poliovirus; (b) a multiple cloning site containing restriction cleavage sites artificially
10 formed at the 5'-end of the genomic nucleotide sequence; and (c) a 3C-protease cleavage site formed between the genomic nucleotide sequence and the cloning site, which comprises performing a mutagenesis of the foreign DNA sequence which is inserted into the multiple cloning site
15 and encodes a multimeric antigen determinant site containing repeated amino acid sequences in order to provide even distribution of G/C content throughout the overall foreign DNA sequence and to avoid repeated nucleotide sequences by use of degeneracy of genetic code.

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17. The method according to claim 16, wherein the mutated foreign DNA sequence encoding multimeric antigen determinant site is prepared by a ligation-free PCR method which comprises the steps of:

25 (i) preparing a plurality of DNA fragment serving as both template and primer and having the following characteristics: (a) the plural primers are designed

by dividing the entire DNA sequence encoding the multimeric antigen determinant site into several fragments with suitable size; (b) the 5'- and/or 3'-ends of the plural primers comprise a complementary sequence to the terminal sequence of other primer; (c) the complementary sequence is 8-20 mer in size and has G/C content of more than 35%; (d) the primers corresponding to both ends in amplification step have a cloning site containing restriction cleavage site;

5 (ii) mixing the primers such that the concentration ratio of the primers corresponding to both ends in amplification step to the other primers is 1:3-1:8;

10 (iii) preparing a full length of the foreign DNA sequence with desired size encoding multimeric antigen determinant site by PCR for 20-40 sec at 92-96°C (denaturation), for 20-40 sec at 25-40°C (annealing) and for 30-55 sec at 68-75°C (extension); and

15 (iv) amplifying the prepared full length of the foreign DNA sequence by PCR for 20-40 sec at 92-96°C (denaturation) and 40 sec - 1 min 10 sec at 68-75°C (annealing and extension).

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18. A method for amplifying a DNA sequence using a ligation-free PCR method, which comprises the steps of:

25 (i) preparing a plurality of DNA fragment serving as both template and primer and having the following characteristics: (a) the plural primers are designed

by dividing the entire DNA sequence of interest into several fragments into several fragments with suitable size; (b) the 5'- and/or 3'-ends of the plural primers comprise a complementary sequence to the terminal
5 sequence of other primer;

(ii) mixing the primers such that the primers corresponding to both ends which is used in amplification step has a higher concentration than the other primers;

10 (iii) preparing a full length of the foreign DNA sequence with desired size encoding multimeric antigen determinant site by PCR for 20-40 sec at 92-96°C (denaturation), for 20-40 sec at 25-40°C (annealing) and for 30-55 sec at 68-75°C (extension); and

15 (iv) amplifying the prepared full length of the foreign DNA sequence by PCR for 20-40 sec at 92-96°C (denaturation) and 40 sec - 1 min 10 sec at 68-75°C (annealing and extension).

20 19. The method according to claim 18, wherein the concentration ratio of the primers corresponding to both ends in amplification step to the other primers is 1:3-1:8.

20. The method according to claim 18, wherein the
25 complementary sequence at the the 5'- and/or 3'-ends of the primers is 8-20 mer in size and has G/C content of more than 35%.

21. The method according to claim 18, wherein the primers corresponding to both ends in amplification step have a cloning site containing restriction cleavage site.

5 22. A recombinant single-stranded RNA virus vector comprising a foreign DNA sequence, characterized in that the foreign DNA sequence is mutated to provide even distribution of G/C content throughout the overall foreign DNA sequence for improving a genetic stability and the
10 recombinant single-stranded RNA virus vector is derived from one selected from the group consisting of a poliovirus recombinant vector, a yellow fever virus vector, a Venezuelan equine encephalitis virus vector, a rubella virus vector and a Cocksackie virus vector.

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23. The recombinant single-stranded RNA virus vector according to claim 22, wherein the foreign DNA sequence mutated has the G/C content of more than 40% and is smaller than 450 bp in size.

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24. A recombinant poliovirus vector comprising a foreign DNA sequence, characterized in that the foreign DNA sequence is mutated to provide even distribution of G/C content throughout the overall foreign DNA sequence for
25 improving a genetic stability and the recombinant poliovirus vector is derived from one selected from the group consisting of poliovirus type 1, poliovirus type 2

and poliovirus type 3.

25. The recombinant poliovirus vector according to claim
24, wherein the foreign DNA sequence mutated has the G/C
5 content of more than 40%.

26. The recombinant poliovirus vector according to claim
24 or 25, wherein the foreign DNA sequence is smaller than
450 bp in size

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27. The recombinant poliovirus vector according to claim
24, wherein the recombinant poliovirus vector is derived
from one selected from the group consisting of Sabin type
1, Sabin type 2 and Sabin type 3.

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28. The recombinant poliovirus vector according to claim
24, wherein the foreign DNA sequence is inserted into a
cloning site containing at least one restriction cleavage
site artificially formed at the 5'-end of the recombinant
20 poliovirus vector.

29. The recombinant poliovirus vector according to claim
28, wherein the cloning site further comprises at its 3'-
direction a protease cleavage site recognized by 3C-
25 protease encoded by a poliovirus genome.